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Recombinant fusion proteins of herpes simplex virus type 1 glycoprotein D; expression in Escherichia coli, purification and immunological properties

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The experiments described in this thesis were started as an attempt to study the immunological properties of herpes simplex virus type 1 (HSV-1) glycoprotein D (gD) using gD fragments produced by recombinant DNA technology. Two different approaches were followed to investigate sequential immunogenic regions of gD. Firstly, restriction enzyme fragments of the gD gene were cloned into a bacterial expression vector. Four different fusion proteins were constructed in this way, containing amino acid residues -5 to 55, -5 to 55 in duplicate, -5 to 161, and -5 to 319 of gD, respectively. To produce fusion proteins with the two largest gD fragments (sequence -5 to 161 and -5 to 319), the original expression vector had to be adapted to a vector coding for a truncated β -galactosidase. Fusion proteins with the truncated β -galactosidase were easier to purify than fusion proteins with the original β -galactosidase, making this vector more suitable to use for immunological studies of protein fragments than the original one. Rabbits were immunized with the gD-fusion proteins and the obtained antisera were investigated for reactivity with synthetic gD-peptides covering the cloned sequences, and in addition for virus neutralizing activity. Several immunogenic sites in the gD-moiety of the fusion proteins were found, viz. amino acid residues 9 to 34, 40 to 54, 70 to 94, 120 to 154, 260 to 274, 280 to 294 and 300 to 314 of gD. HSV-neutralizing antibodies appeared earlier after immunization with the tandem fusion protein, containing the sequence -5 to 55 in duplicate, than after immunization with the other three fusion proteins. Immunizations with this fusion protein elicited higher anti-gD-peptide antibody titers than immunizations with the other three fusion proteins. These results suggest that a different presentation of an antigenic region of a protein may enhance the immune response to this region and that relatively short gD fragments are equally effective in eliciting neutralizing antibodies as longer fragments.

Secondly, instead of well-defined restriction enzyme fragments, randomly-generated fragments, produced by DNase-treatment of the gD gene were cloned into the expression vector. The resulting random expression library was screened with a human HSV-positive antiserum. In addition, a set of overlapping synthetic gD-peptides covering nearly the complete gD sequence was used.

regions, recognised by the human immune system, viz. residues 10 to 24, 260 to 274, 300 to 314 and 340 to 369. Combining the results of both approaches the following regions of gD were found to be immunogenic by studies with the gD-fusion proteins: 9 to 34, 40 to 54, 70 to 94, 120 to 154, 260 to 274, 280 to 294, 300 to 314, and 340 to 369. Residues 9 to 34, 260 to 274, and 340 to 369 overlap epitopes described by Cohen and Eisenberg (1, 2); the other regions were not identified before. Whether these regions are antigenic in native gD and whether they are capable of eliciting virus neutralizing antibodies remains to be investigated.

This study indicates that a limited number of clones expressing different fragments of gD in combination with synthetic peptides covering the gD sequence are very suitable for the determination of sequential antigenic regions. It will be interesting to investigate the immunological potentiation of fusion proteins with more than two copies of sequence -5 to 55 of gD, since immunization with a fusion protein containing twice the sequence -5 to 55 showed a tendency to result in higher antibody titers than immunizations with the other fusion proteins. Furthermore, it has to be determined whether the relatively short fusion proteins (sequence -5 to 55 of gD) are equally effective in inducing protection against an HSV-1 challenge as the longer fusion proteins (sequence -5 to 161 and -5 to 319 of gD).

The gD-fusion proteins may also be suitable tools in localization-studies of T-cell epitopes which are necessary for the induction of cellular immunity. Both B- and T-cell epitopes may be required for the establishment of long-lasting protective immunity.

Finally, expression of gD in a eukaryotic expression system will be necessary to investigate structural antigenic sites of gD and their role in the production of virus neutralizing antibodies. The results of such investigations may eventually result in a subunit vaccine against HSV-1.